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Escherichia coli Vaccine and Laying Hens Mortality After A Heat Stress Challenge in Tropical Climate

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Abstract: The objective of the present research was to evaluate a vaccine against *E. coli* in laying hens in tropical climates and its response to heat stress challenge. About 47880 Bovans White laying hens were assigned at two treatments: 24.806 were vaccinated (sprayed) against *Escherichia coli* and 23 074, the control treatment were not vaccinated. Two applications were made during breeding, the day 1 and week 12. At 16 weeks were transferred form the breeding farm to the layer farm and were distributed at random in a layer house and identified. The daily mortality in both treatments was recorded. A t-test is performed to determine differences between daily mortality rates, differences were found for the three stages: 16-23 weeks ($p<0.05$) (0.05+0.04 vs 0.08+0.12) from 23-1 to 34 ($p<0.0001$) (0.04+0.02 vs 0.07+0.03) and 34-1 to 46-2 ($p<0.0001$) (0.04+0.01 vs. 0.08+0.04). Mortalities in this flock were 14.384% for unvaccinated and 8.224% in vaccinated. Regression analysis were performed for cumulative mortalities before and after a heat stress event, finding that the trend lines have different slopes for birds vaccinated and unvaccinated before and after the event. Vaccination against *E. coli* showed a lower mortality in the study conditions and a relation whit heat stress event.

Key words: *E. coli* vaccine, egg layers, mortality, temperature, heat stress, THI

INTRODUCTION

In tropical regions, weather conditions caused a sharp decline in yields of laying hens and in many cases high mortality. Climatic factors such as maximum and minimum temperatures and relative humidity are important in production systems with uncontrolled environment booths (open houses); effect is lessened in controlled environment systems (Czarick, 1990). The greatest adverse effect caused by high humidity as it has been shown that hens can be handled better and efficiently with high temperatures, if the relative humidity is maintained within permissible ranges or below 50% (Ortiz *et al*., 2006). Feed intake decreases during heat stress in laying hens and consequently has a negative impact on production and egg size, this factor is mainly influenced by climatic conditions and regulated by metabolic processes including energy, protein and amino acids (Ortiz *et al*., 2006). Mortality in laying hens in tropical areas is multifactorial response, Malavet Bustos found a mortality of 13.2%, corresponding (%) with reproductive disorders (25.83), cannibalism (25.0), nephritis (17.29), asphyxia (14.13), leukemia (5.55), Marek (4.45), internal bleeding (3.49), colibacillosis (1.38), renal hematites (1.60), other malignancies (1.28), mycotoxins (1.0) and avian edema syndrome (0.60). For the conditions of the Yucatan Peninsula, in Mexico, Ortiz *et al*. (2006) indicate that the maximum temperature and relative humidity are factors in the increased mortality and affect feed consumption. For every Celsius degree increase or decrease in temperature, affects the consumption at a rate of 3.36% which represents 3.19 g day^{-1}. They also found that the main factors of mortality are not diagnosed (43.42%) followed by cannibalism (24.66%) and cage fatigue (19.52%). *E. coli* is a normal inhabitant of the intestines of birds, so it is always present in the rearing of turkeys and broilers. Although, many strains of *E. coli* virulence have not cause disease. *E. coli* is a secondary infection after a first viral or environmental attack. Along with other factors may determine the severity of the reaction to vaccination of chickens against Newcastle disease and bronchitis and has a minor role in the mycoplasmosis. Environmental agents such as ammonia, CO_2 and dust produce damage to the cilia of the respiratory tract causing the onset of colibacillosis. We found that it is a major cause of mortality in shipments of live birds and that 2.4% of mortality per trip is due to problems related to *E. coli* and complicated with

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increasing stress. The implementation of vaccination programs can be a tool to reduce the use and subsequent resistance to antibiotics. In 2006 was introduced to the market a live vaccine to control airsacculitis and peritonitis in birds. The vaccine is a virulent and genetically modified to protect against several strains including 078 with the intention of reducing the impact on infections caused by *E. coli*. A vaccine is recommended for use in mass of at 1-3 days old for birds with long periods of production and laying hens, a second application (booster) between 12-14 weeks of age. The effect of vaccine in laying hens is expected to reduce mortality, morbidity and loss in egg production associated with peritonitis by the agent. The objective of this study was to evaluate the effect of vaccination against *E. coli* in the mortality of laying hens and after a heat stress challenge.

MATERIALS AND METHODS

The study was conducted in the state of Yucatan, Mexico with a height of 6 m over the sea level and AW0 prevailing climate. About 47880 laying hens from Bovans White line were assigned at to two rearing treatments: 24.806 were air vaccinated against *E. coli* strain 078 and 23.074 unvaccinated. The birds were vaccinated by spraying at day one following a booster application at 12 weeks old. For spray vaccination, the vaccine vials were removed the seal and plastic lid then fill the vial halfway with cold distilled water placed the rubber stopper and shook until dissolved. Hydrated vaccine was dissolved in a clean container by adding distilled water as directed by the vaccination machine. The calculation of the vaccine rate according Nuno Ayala. All susceptible birds were vaccinated at the same time and prevented the exchange of material between vaccinated and unvaccinated birds. At 16 weeks, birds were removed from the breeding farm to egg layer farm position. The birds were randomly allocated and identified in the layers house for position. At the farm stand, the dead birds in the flock were collected and counted according to the treatment.

Daily mortality was divided into 3 periods for the flock, start-up phase of 16-23 weeks old at the end of which there was an event of high heat stress and mortality, intermediate stage of 23-1 to 34 weeks old and final phase of lot 34-1 to the 46-2 weeks old. After checking the normal distribution, data were analyzed by a paired Student's t-test averages with comparisons of mortality per day of age. There was also a regression between temperature and mortality as well as Temperature and Humidity Index (THI) and mortality. Finally for the cumulative mortalities were performed a linear regression analysis in groups of vaccinated birds before and after heat stress. Statistical analysis was performed using the program Prism 5.0.

RESULTS

The difference found at the end of the flock in cumulative mortality can be attributed to the effect of the vaccine (Table 1), since in the three study periods in daily mortality significant differences between treatments were found, favoring the vaccinated birds. The daily mortality in vaccinated birds is lesser than in no vaccinated (Table 2). As for the linear regression between daily mortality and maximum temperature of the day, it was found that throughout the production flock presents a similar slope in the figures but the points of origin and end are highly significant different. The fit was better when using the data that from the maximum temperature instead of THI.

In the present study found that temperature has a value of association (R^2) greater and lower residual regressions performed with the calculated THI (Table 3). Unlike what was reported in the literature, the maximum temperature was an effective determinant of mortality more than the air moisture content and temperature expressed as THI but this effect in the study can be explained physically, since the higher air temperature, the lower relative humidity and situations in which there is a high relative humidity with high temperature corresponds

Table 1: Cummulative mortality in Bovans White hen layers, vaccinated or no vs *E. coli*

Treatments	No. of birds	Total mort.	Cummulative mort. (%)	Live
No vaccinated	23,074	3319	14.384	19755
Vaccinated	24,806	2040	8.224	22766

Table 2: Daily mortality in Bovans White hen layers vaccinated or no vs *E. coli*

	16-0 to 23-0 weeks			23-1 to 34-0 weeks			34-1 to 46-2 weeks		
Mortality	Vac	No Vac	Dif.	Vac	No Vac	Dif.	Vac.	No vac	Dif
Minimum	0.000	0.000	-	0.000	0.010	-	0.013	0.000	-
Maximun	0.243	0.690	-	0.101	0.140	-	0.088	0.180	-
Mean	0.052	0.078	*	0.038	0.066	***	0.035	0.077	***
SD	0.040	0.118	-	0.018	0.030	-	0.012	0.038	-

Vac Vaccinated, No Vac No Vaccinated, * = $p<0.05\%$; ***$p<0.0001\%$

Table 3: Daily mortality and Temperature Humidity Index (THI) or maximum temperature regression

Statistical analysis	Maximum temperature	THI
Y if X = 0.0	-0.07912±0.024	-0.1560±0.065
X if Y = 0.0	23.92	24.55
1/slope	302.3	157.4
r^2	0.10620	0.05603
Sy.x	0.02302	0.06253
p-value	<0.0001	<0.00050

Table 4: Cummulative mortality regression values for vaccinated and no Bovans White Hen Layers, before and after a heat stress

	Before heat stress		After heat stress	
Statistical analysis	Vac.	No vac.	Vac.	No vac.
Y if X = 0	-0.1411±0.026	-0.07502±0.024	2.756±0.0081	3.763±0.026
X if Y = 0	2.59	1.22	-80.17	-56.43
1/slope	18.32	16.25	29.09	14.99
r^2	0.986	0.991	0.999	0.997
Sy.x	0.09	0.08	0.05	0.16
p-value	<0.0001	<0.0001	<0.0001	<0.0001

to exceptional cases (Czarick, 2006). After heat stress, the cumulative mortality of no vaccinated birds has a slope of higher mortality than the vaccinated ($p<0.01$), so heat stress is influencing mortality in the flock during the production cycle and the difference between mortalities can be explained by the vaccine against *E. coli*. When performing linear regressions of the cumulative mortality over the age of the birds in days, there was a linear response as shown in Table 4. There were statistical differences between the four regressions because the slope is different between them however, the slope of the cumulative mortality after the incident of heat stress has a tendency to lower mortality as it passes time.

DISCUSSION

Evidence that vaccination could be successful dating back <20 years, Gyimah *et al.* (1986) used a multivalent vaccine prepared with *E. coli* serotype 01, 02 and 078 and administered subcutaneously, effectively protect chickens for 4 weeks with a difference between 8 and 24% in mortality when compared with unimmunized control groups. However, vaccinated chickens showed very mild gross lesions in air sacs and pericardium and liver, eliminated *E. coli* more efficiently showing that a multivalent vaccine protects birds against active infections. Using genetic engineering, Kwaga *et al.* (1994) transferred a mutation generated in the laboratory to virulent strains of field, inserted into the operon Cerabar and the degree of attenuation was evaluated in chickens of 1 day old using 100 times the average dose lethal and earning lower mortality in the chickens vaccinated orally. Turkeys vaccinated at 4 weeks were completely protected against challenge indicating that the mutants can be effectively used as live vaccines preventing colibacillosis.

The effectiveness of immunization against *E. coli* may depend on other factors, Heller *et al.* (1992) in lines selected on antibody response to immunization with nonpathogenic strains of *E. coli* found higher titers of antibodies to the vaccine 20 days after concluding in a later research the same team (Leitner *et al.*, 1994) that genetic and environmental variables influence the animal's resistance to infectious diseases. They also found maternal effects on egg chicken lines. Using crosses between lines selected for humoral immune responses found that *E. coli* can provide separate estim ates of maternal and paternal effects finding a more marked effect of the maternal lines which indicates the possibility of different responses between the lines.

Heat stress is known to affect consumption, production, egg weight and egg weight and shell quality in hen layers in tropical climates (Ortiz *et al.*, 2006) however, not only in production reflected the poor performance, environmental stress can depress the immune function of birds and an effective cell-mediated immunity (Zulkifli *et al.*, 1994). Heat stress induces numerous hormonal responses in warm-blooded species. Of particular interest are the thyroid hormones 3,5,3´-Triiodothyronine (T3) and Thyroxine (T4). T3 is converted from T4 by deiodinases in various tissues such as liver, kidney, pituitary gland and brown adipose tissues (Bianco and Larsen, 2005). The T3 produced in these tissues is released into the plasma and becomes the sthece for intracellular T3 in skeletal muscle. As animals change their thyroid hormone level in response to temperature, the change could affect Ca^{2+} regulation in skeletal muscle (Everts, 1996). Since an abnormally high Ca^{2+} content during muscle contraction is responsible for muscle hypermetabolism and the subsequent postmortem meat quality defect, changes in thyroid hormone level may trigger a cascade of signals leading to inferior meat quality development when birds are subjected to heat stress. Normally, warm-blooded animals respond to ambient temperature by decreasing thyroid hormone secretion rate as ambient temperature increases and vice versa (Silva, 2003; Chiang *et al.*, 2008). Also the phagocytic potential of macrophages from chicken decreases during exposure to heat stress (Miller and Qureshi, 1992).

Guo *et al.* (1998) indicate that high temperatures can affect the development of immune organs. Stress can cause changes in the number of leukocytes (relationship heterophils/lymphocytes), a situation that for a short period can increase resistance to infections with *E. coli* while more prolonged stress and reduced consumption leading to reduction of lymphocytes and atrophy of lymphoid organs (Rosales, 1994). However, the results of this study suggest that a short term stress alters the patterns of mortality in way related to the ability to confront the *E. coli*.

CONCLUSION

In the present study we found a positive effect of vaccination against *E. coli* in daily mortality when compared with a control group without vaccine and mortalities doe to heat stress are less in vaccinated birds. Under the conditions of the study the temperature data can be a valuable predictor of mortality that can occur in natural ventilation hen layers farms.

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